

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Kvist et al. **GROUP:** 4970
SERIAL NO: 10/815,045 **EXAMINER:** Carolyn A. Paden
FILED: March 30, 2004
FOR: PROCESS FOR FRACTIONATION OF OILSEED PRESS
CAKES AND MEALS

Mail Stop Patent Application
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
Sir:

RESPONSE

This is in response to the Office Action mailed on September 6, 2007.

Claims 1-7, 28 and 29 have now been rejected under 35 U.S.C. §103(a) as being unpatentable over the Klockeman thesis, in view of Reverso, U.S. Patent No. 6,103,516.

The Examiner's rejection is respectfully traversed.

The Applicants' invention is directed to a process for the wet fractionation of oil seed press cake and/or meal. This process comprises dispersing oil seed press cake or meal in water and subjecting it to a combined treatment of wet milling, enzymatic treatment by using one or a combination of the following enzymes: beta-glucanase, xylanase, hemicellulase, arabinase and pectinase and heat, followed by a sequential fractionation at an elevated temperature using centrifugal forces and size exclusion (ultrafiltration) so as to yield one or more fibrous-rich fractions, at least three different protein-rich fractions, optionally an oil-rich fraction, a sugar-rich fraction and a phytate-rich fraction, The final step consists of drying or partial evaporation of the above-said fractions.

The Klockeman thesis describes an extraction method which maximizes protein recovery from canola meal. The differences between the Klockeman method and that of the claimed invention include the fact that the method in Klockeman uses a different starting material, namely canola meal which has been hexane defatted and expeller treated (see p. 30, 1st paragraph under Materials and Methods). The present invention uses rapeseed cake which has not been defatted using solvent extraction but only processed by expeller pressing (definition of oilseed cake p. 1, l. 22-23). However the present method can also be used on oilseed meal (Example 4).

In Klockeman different methods (solubility in basic solutions, NaCl solutions, dilute ethanol and water respectively) for extracting proteins were tested, but maximum protein extractability was obtained with a 5% w/v meal ratio, 0.4 % w/v NaOH extract for 60 min at 180-200 rpm (Figure 3, and p 33, l 5-6) and at room temperature (p. 31, l. 16). After the extraction, the soluble material is separated from the soluble extract by centrifugation and glacial acetic acid is added to the soluble extract where after the proteins precipitate from the solution. The precipitated proteins are separated by centrifugation and washed in distilled water (p. 31, l. 16-23). The protein recovery is 87.5 % upon precipitation with acetic acid (Abstract p. 29, and p. 33, l. 9). There is no mention of other fractions such as oil, fibers or phytate.

In the present invention the press cakes are treated with specific carbohydrate-degrading enzymes in combination with wet milling at temperatures from 20-90 °C, preferably from 30-50 °C and a pH from 4-6.5 (p.2, lines 6-10) for 3 hours (Example 1, l. 13). The resulting hydrolysate is heated to 50-90 °C (95 °C in Example 1) and separated into solubles and precipitate by centrifugation. The solubles are suspended in water and again centrifuged to give five layers; two top oil layers, two bottom protein-fiber layers and one

middle soluble layer, said middle soluble layer being further separated into a sugar and protein fraction by ultra filtration. When the method of the present invention is used for the press cake, the overall protein extraction was 71.3 % (Example 1) or 83 % (Example 3), and for oilseed meal 94.2 % (Example 4).

The technical effect associated with these differences is that the protein fractions provided by the present method are free of alkali or other chemicals which always are present in trace amounts in the final product when chemicals are used as precipitating agents: Additionally, the present invention provides a process which easily separates the high value protein, fat, sugar, fibre, and, not the least, phytate fractions from each other, without having to use additional precipitation agents such as exogenous divalent cations or chelating agents.

The invention of claim 1 solves this problem by providing a method for fractionating oil seed press cakes (or meal) into one or more fibrous rich fractions, at least three different protein rich fractions, optionally an oil fraction, a sugar-rich fraction and a phytate-rich fraction, by using a combination of carbohydrate-degrading enzymes combined with wet milling and subsequently separating the different fractions from each other without using precipitation or chelating agents.

If the skilled person faced with the problem to fractionate the oilseed press cake (or meal) into one or more fibrous rich fractions, at least three different protein rich fractions, optionally an oil fraction, a sugar-rich fraction and a phytate-rich fraction, without using the chemicals disclosed in the Klockeman thesis, (that is: basic solutions, NaCl solutions, dilute ethanol or glacial acetic acid) were to look to the U.S patent 6,103,516 by Reverso, he would not arrive at the claimed subject matter.

Reverso discloses a method for extracting oil from oleaginous plants, using at least one carbohydrate-degrading enzyme. However, this method is targeted at releasing oil from

the cytosol of the seeds or caryopsides of the plants by breaking up the saccharide polymers that are present in the external integument. For plants such as olives there is a need to break up at least two polymeric components: hemicellulose and pectin but for other seeds such as soya or maize etc. a single enzyme is sufficient (Reverso col. 1, l. 56- col. 2, l. 20). In the method by Reverso the enzyme/enzymes are added to the solid oleaginous parts at temperatures between room temperature and approx 35 °C under agitation at a pH between 5.5 and 7.8 for 1-2 minutes. After this the oil is separated by, for example, centrifugation (col.24, l. 22-35).

There are a couple of reasons the method of Reverso cannot be combined with the method disclosed by Klockeman. First of all, Reverso uses enzymes targeted at opening a path into the cytosol only. Consequently, a treatment time of a few minutes suffice for releasing the oil. This enzyme treatment will however not degrade the oilseed cakes (or meals) to provide all the other fractions disclosed in the present invention. Secondly, the present invention uses several different enzymes that will target different parts in the cell wall simultaneously. The beta-glucanase breaks down cellulose, xylanase and hemicellulose break down hemicellulose and the arabinase and pectinase will target pectic substances. These enzymes are used combined with wet milling and heat for 3 hours. Thus, with this combination of enzymes and long treatment time the cell walls will be completely degraded and the proteins (and other valuable fractions) contained in the cell walls and the cytosol are completely released, and can easily be recovered by separating methods such as centrifugation or filtration, without using precipitating agents. Due to this “incomplete” degradation of the cell wall, which will not release the proteins bound in the cell wall, the protein fractions, protein content or protein composition obtained by the Reverso method cannot possibly be the same as after the treatment in the present invention.

In view of the foregoing, the Applicants believe that the independent claims and the claims dependent therefrom are in proper form. The Applicants respectfully contend that the teachings of the Klockeman thesis, in view of Reverso U.S. Patent No. 6,103,516 do not establish a *prima facie* case of obviousness under the provisions of 35 U.S.C. §103(a). Thus, claims 1-3, 5-7 and 28-29 are considered to be patentably distinguishable over the prior art of record and should be allowed.

The application is now considered to be in condition for allowance, and an early indication of same is earnestly solicited.

The Commissioner is authorized to charge Deposit Order Account No. 19-0079 for any fees that may be required.

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'Arlene J. Powers', is written over a horizontal line.

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